

RESEARCH ARTICLE

Seasonal and geographical variation in heat tolerance and evaporative cooling capacity in a passerine bird

Matthew J. Noakes¹, Blair O. Wolf² and Andrew E. McKechnie^{1,*}

ABSTRACT

Intraspecific variation in avian thermoregulatory responses to heat stress has received little attention, despite increasing evidence that endothermic animals show considerable physiological variation among populations. We investigated seasonal (summer versus winter) variation in heat tolerance and evaporative cooling in an Afrotropical ploceid passerine, the white-browed sparrow-weaver (*Plocepasser mahali*; ~47 g) at three sites along a climatic gradient with more than 10°C variation in mid-summer maximum air temperature (T_a). We measured resting metabolic rate (RMR) and total evaporative water loss (TEWL) using open flow-through respirometry, and core body temperature (T_b) using passive integrated transponder tags. Sparrow-weavers were exposed to a ramped profile of progressively higher T_a between 30 and 52°C to elicit maximum evaporative cooling capacity ($N=10$ per site per season); the maximum T_a birds tolerated before the onset of severe hyperthermia ($T_b \approx 44^\circ\text{C}$) was considered to be their hyperthermia threshold T_a ($T_{a,HT}$). Our data reveal significant seasonal acclimatisation of heat tolerance, with a desert population of sparrow-weavers reaching significantly higher T_a in summer ($49.5 \pm 1.4^\circ\text{C}$, i.e. higher $T_{a,HT}$) than in winter ($46.8 \pm 0.9^\circ\text{C}$), reflecting enhanced evaporative cooling during summer. Moreover, desert sparrow-weavers had significantly higher heat tolerance and evaporative cooling capacity during summer compared with populations from more mesic sites ($T_{a,HT} = 47.3 \pm 1.5$ and $47.6 \pm 1.3^\circ\text{C}$). A better understanding of the contributions of local adaptation versus phenotypic plasticity to intraspecific variation in avian heat tolerance and evaporative cooling capacity is needed for modelling species' responses to changing climates.

KEY WORDS: Acclimatisation, Heat stress, Hyperthermia, Evaporative water loss, Arid-zone birds

INTRODUCTION

Birds inhabiting hot environments where air temperature (T_a) regularly exceeds normothermic body temperature (T_b) face physiological and behavioural challenges related to the avoidance of lethal hyperthermia and maintenance of water balance (Cade, 1965; Dawson and Bartholomew, 1968; Williams and Tieleman, 2005). These challenges are often manifested as consequential trade-offs affecting behaviour, body condition and reproductive decisions (e.g. du Plessis et al., 2012; Smit et al., 2013; Tieleman et al., 2008), which are likely to be strongly affected by the increases in maximum T_a ($T_{a,max}$) and

duration and frequency of extreme heat waves predicted to occur during the 21st century as a result of anthropogenic climate change (Intergovernmental Panel on Climate Change, 2012).

Reports of intraspecific variation in physiological traits are providing increasing evidence that the thermal physiology of endotherms is far more flexible than was previously recognised, and that conspecific populations may vary substantially in their thermoregulatory physiology, even over relatively small climatic gradients (e.g. Glanville et al., 2012; Smit et al., 2013). Understanding intraspecific variation in heat tolerance and evaporative cooling capacity, and the relative contributions of local adaptation versus phenotypic plasticity to inter-population differences, is directly relevant to testing the assumption implicit in climate-envelope modelling studies that species cannot occupy habitats hotter than those within which they currently occur (Boyles et al., 2011; Pearson and Dawson, 2003).

Evaporative water loss (EWL) is the only avenue of heat dissipation in birds when T_a exceeds normothermic T_b , and many birds have the capacity to maintain T_b significantly below T_a when necessary (Crawford and Schmidt-Nielsen, 1967; Whitfield et al., 2015; Williams and Tieleman, 2005). The relative importance of respiratory and cutaneous evaporation for thermoregulation at high T_a varies among taxa, with increases in respiratory evaporative water loss by panting being the predominant mechanism in all passerine birds investigated to date (Ro and Williams, 2010; Tieleman and Williams, 2002; Wolf and Walsberg, 1996). Panting requires an increase in ventilation rate (Calder and Schmidt-Nielsen, 1967; Dawson, 1982), and as T_a increases above the upper critical limit of thermoneutrality (T_{uc}), a concomitant increase in metabolic rate is typically observed (e.g. Ambrose et al., 1996; Tieleman et al., 2002a; Trost, 1972; Williams, 1999).

Little is known about how heat tolerance and evaporative cooling capacity vary among and within avian species, as the majority of studies have focused on resting metabolic rate (RMR) and/or EWL at $T_a < T_b$ (e.g. Dawson, 1982; Tieleman et al., 2002a; Williams, 1996). For example, a review comparing the physiological responses of 102 avian species demonstrated that desert birds have lower EWL at moderate T_a than mesic species (Williams, 1996), a response thought to be adaptive by conserving water and reducing heat production in birds inhabiting hot environments with scarce drinking water and low primary productivity (Tieleman et al., 2002a; Williams and Tieleman, 2000). Recently, Whitfield et al. (2015) quantified variation in the upper limits of heat tolerance and evaporative cooling capacity among three ploceid passerines varying approximately fourfold in body mass (M_b). These authors found that the maximum T_a tolerated during acute heat exposure was positively related to M_b , ranging from ~48°C in the 10 g scaly-feathered weaver (*Sporopipes squamifrons*) to ~54°C in the 40 g white-browed sparrow-weaver (*Plocepasser mahali*). In one of the few studies to examine intraspecific variation in variables related to heat tolerance, Trost (1972) found that the physiological responses

¹DST-NRF Centre of Excellence at the Percy FitzPatrick Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa. ²UNM Biology Department, University of New Mexico, MSC03-2020, Albuquerque, NM 87131-0001, USA.

*Author for correspondence (aemckechne@zoology.up.ac.za)

List of symbols and abbreviations

BMR	basal metabolic rate
CEWL	cutaneous evaporative water loss
EHL	evaporative heat loss
EWL	evaporative water loss
M_b	body mass
MHP	metabolic heat production
RMR	resting metabolic rate
T_a	air temperature
$T_{a,HT}$	hyperthermia threshold air temperature
$T_{a,max}$	maximum air temperature
$T_{a,min}$	minimum air temperature
T_b	body temperature
TEWL	total evaporative water loss
TNZ	thermoneutral zone
T_{uc}	upper critical air temperature

of desert and mesic horned larks (*Eremophila alpestris*) were indistinguishable at $T_a < 45^\circ\text{C}$, but at $T_a = 45^\circ\text{C}$ the desert population had significantly lower total EWL (TEWL; ~18% lower) and RMR (~32% lower) than their mesic conspecifics.

Seasonal acclimatisation of physiological responses is a form of phenotypic flexibility (*sensu* Piersma and Drent, 2003) and has been well studied at thermoneutral and low T_a . In temperate-zone birds, well-documented examples involve the up-regulation of basal metabolic rate (BMR) and summit metabolism during winter, compared with summer (reviewed by McKechnie, 2008; McKechnie and Swanson, 2010; Swanson, 2010). In contrast, we are aware of only one study in which seasonal acclimatisation of avian physiological responses at $T_a > T_{uc}$ was reported (Tieleman et al., 2002b). These authors demonstrated that captive-bred houbara bustards (*Chlamydotis macqueenii*) had significantly higher RMR at $T_a = 35$ and 50°C (~23% higher), and TEWL at $T_a = 35^\circ\text{C}$ (~46% higher) during winter than in summer (Tieleman et al., 2002b). It has been hypothesised that greater phenotypic flexibility confers adaptive advantages to organisms that inhabit temporally heterogeneous environments (Schlichting and Pigliucci, 1998). A number of studies involving short-term thermal acclimation experiments were designed to address whether a correlation exists between the magnitude of phenotypic flexibility of avian physiological responses and environmental aridity (Cavieres and Sabat, 2008; Tieleman et al., 2003b; Tieleman and Williams, 2002), but these focused on responses at thermoneutral T_a values.

The first step towards understanding the roles of local adaptation and phenotypic plasticity is to quantify variation among populations along climatic gradients. We therefore investigated seasonal and geographical variation in thermoregulatory responses to high T_a in a widespread southern African passerine bird, the white-browed sparrow-weaver (*Plocepasser mahali*). Three populations were chosen along a climatic gradient ranging from areas where $T_{a,max}$ is well below normothermic T_b (two mesic sites), to a desert site where $T_{a,max}$ routinely exceeds normothermic

T_b in summer. We hypothesised that heat tolerance and evaporative cooling capacity vary among populations in a manner correlated with $T_{a,max}$, and that seasonal acclimatisation of these physiological variables occurs at sites with pronounced seasonal variation in $T_{a,max}$. Specifically, we predicted that evaporative cooling is more efficient in individuals that routinely experience T_a exceeding normothermic T_b than in those that do not, with greater evaporative cooling efficiency associated with more gradual increases in T_b , EWL and/or RMR at $T_a > T_b$. We define the efficiency of evaporative cooling as the ratio of evaporative heat loss to metabolic heat production (EHL/MHP). We also predicted that sparrow-weavers from hot regions tolerate higher T_a during acute heat exposure compared with conspecifics from cooler sites.

MATERIALS AND METHODS**Study species and sites**

The white-browed sparrow-weaver [*Plocepasser mahali* (Smith 1836); hereafter, sparrow-weaver] is a ploceid passerine widespread across southern Africa (du Plessis, 2005). We examined thermoregulation in sparrow-weavers during the austral winter (July–August 2013 and 2014) and summer (January–February 2014) at three study sites in South Africa that vary in seasonal temperature extremes: one arid site near Askham in the Kalahari Desert (Northern Cape Province), and two mesic sites at Frankfort (Free State Province) and Polokwane (Limpopo Province; Table 1). These sites were all within the distributional range of the subspecies *P. mahali mahali* (du Plessis, 2005).

Climate data were obtained from the South African Weather Service using the weather station closest to each study site. There are weather stations at Polokwane (~9.1 km north of our study site) and Frankfort (~1.4 km away), but the nearest station to Askham is at Twee Rivieren (~62 km northwest; Table 1). For each site, we extracted mean daily $T_{a,max}$ and minimum T_a ($T_{a,min}$) values over the hottest summer month (January) and coldest winter month (July) during the season in which we collected data (Table 1). The highest $T_{a,max}$ values occurred at Twee Rivieren (~Askham; ~10°C higher than at Polokwane), as did the most pronounced seasonal variation in $T_{a,min}$ and $T_{a,max}$ values (~14°C higher $T_{a,max}$ in summer than in winter; Table 1). There was also pronounced seasonal variation in T_a extremes at Frankfort, but with comparatively milder summer $T_{a,max}$ values (Table 1). In contrast, Polokwane had relatively mild summers and winters (Table 1).

Sparrow-weavers were typically caught at night using two small nets mounted on aluminium poles placed over the entrances of roost nests. A few birds were caught during the day using mist nets or spring traps baited with mealworms. To avoid trapping reproductive individuals, we did not catch birds over the peak egg-laying period for *P. mahali* (November–December; du Plessis, 2005), and avoided catching sparrow-weavers from breeding nests. Physiological data were collected at the various study sites, and birds were housed in cages constructed of plastic mesh and shade cloth (~1.5 m³) for no more than 48 h prior to measurements.

Table 1. Mean daily air temperature minimum ($T_{a,min}$) and maximum ($T_{a,max}$) during the hottest summer month (January) and coldest winter month (July)

Study sites	Daily $T_{a,min}$ (°C)		Daily $T_{a,max}$ (°C)		Location	
	Summer	Winter	Summer	Winter	Study site	Weather Station
Askham	19.7±3.7	-0.3±5.4	37.7±3.7	23.8±2.8	26°59'S, 20°51'E	26°28'S, 20°36'E
Frankfort	14.0±2.1	-2.5±3.2	29.3±2.5	19.7±2.3	27°16'S, 28°29'E	27°16'S, 28°29'E
Polokwane	17.2±2.1	4.3±1.2	26.9±2.1	21.2±3.5	23°56'S, 29°28'E	23°51'S, 29°27'E

Data (means±s.d.) are for three study sites in South Africa, calculated from weather data obtained from South African Weather Service stations.

The diet of *P. mahali* includes insects, seeds, fruits and fleshy leaves, and in the Kalahari Desert they eat mostly insects (~80%; du Plessis, 2005). While in captivity, birds were provided with water and a wild bird seed mix *ad libitum*, as well as giant mealworms (~5 per bird per day). Individuals were sexed by bill colour (du Plessis, 2005), and a Scout *Pro* Balance scale (SP602US, Ohaus, Pine Brook, NJ, USA) was used to measure M_b to 0.01 g. Sparrow-weavers were released at their site of capture after data collection.

All experimental procedures were approved by the Animal Ethics Committee of the University of Pretoria (protocol EC030-13) and the relevant permitting authorities in Northern Cape, Limpopo and Free State provinces.

Gas exchange and temperature measurements

We measured CO_2 production (ml min^{-1}) and TEWL (mg min^{-1}) using an open flow-through respirometry system, and core T_b of sparrow-weavers using temperature-sensitive passive integrated transponder tags, using the same experimental setup as Whitfield et al. (2015). All sparrow-weavers were placed individually in airtight respirometry chambers constructed from 4 l clear plastic containers (Lock & Lock, Seoul, South Korea). Relatively high flow rates (2–20 l min^{-1}) were used, and were continuously monitored and adjusted during data collection to ensure that water vapour partial pressure within the chambers always remained low (<0.31 kPa), while also maintaining differences in $[\text{CO}_2]$ and $[\text{H}_2\text{O}]$ between incurrent and excurrent air sufficient for accurate measurements. All equipment was calibrated and passive integrated transponder tags injected into birds as described by Whitfield et al. (2015).

Experimental protocol

To quantify heat tolerance and maximum evaporative cooling capacity in *P. mahali* in a manner facilitating comparisons among and within populations, we measured gas exchange rates and T_b of sparrow-weavers individually during their active phase (day-time) using the standardised protocol described by Whitfield et al. (2015; modifications described below). We exposed birds to a ramped T_a profile of progressively higher T_a values (between 30 and 52°C) in a stepwise fashion, with individuals being maintained at constant T_a values for a period of ≥ 10 min (mean exposure time per T_a , 15.2 ± 4.6 min; calculated from a subset of 42 of 240 data files), before increasing T_a to the next setpoint. Different individuals were used for measurements at $30^\circ\text{C} \leq T_a \leq 38^\circ\text{C}$ and $T_a \geq 40^\circ\text{C}$, with a sample size of $N=10$ per site per season for each of these two T_a ranges (each bird was only exposed to a given T_a once). In the lower T_a range, individuals were exposed to constant T_a values of 30, 34, 36 and 38°C, and in the higher T_a range, data were collected from $T_a=40^\circ\text{C}$ upwards in 2°C increments until birds became hyperthermic. The behaviour of birds during trials was monitored as described by Whitfield et al. (2015), but we did not present or analyse behavioural observations as there were seldom enough records of active birds to enable reliable comparisons, and because of the difficulty of quantifying and interpreting behavioural responses. Trials were ended when birds showed signs of distress (this occurred in only three cases) or in calm birds when their T_b exceeded 44°C. This value was chosen as Whitfield et al. (2015) found that $T_b=44$ to 45°C is close to the critical thermal maximum for three ploceid passerines (including *P. mahali*), and pilot studies demonstrated that sparrow-weavers at our more mesic sites became behaviourally distressed at $T_b>44^\circ\text{C}$. The T_a at which each bird reached $T_b>44^\circ\text{C}$ (actual $T_b=44.3^\circ\text{C}$ after calibration) was thus considered the

hyperthermia threshold T_a ($T_{a,HT}$) for that individual in the present study. If birds had to be removed earlier for reasons other than severe hyperthermia (e.g. power outages or intermittent passive-integrated transponder tag reception), the data were excluded from $T_{a,HT}$ analyses.

Data analyses

Data were corrected for drift in $[\text{CO}_2]$ and $[\text{H}_2\text{O}]$ baselines using the relevant algorithms in Expedata data acquisition and analysis software (Sable Systems, Las Vegas, NV, USA). For each bird, the 5 min sample period with the lowest average $[\text{CO}_2]$ at each T_a was assumed to be representative of resting values, and behavioural observations were used to verify that birds were calm during this period. In a few cases, birds were not calm for a full 5 min at a given T_a , and thus all data from these birds at that T_a were discarded. Whole-animal rate of CO_2 production (\dot{V}_{CO_2}), RMR (W) and TEWL values were calculated as described in Whitfield et al. (2015), except that a respiratory exchange ratio of 0.85 was assumed (representative of a metabolic substrate consisting of a mix of carbohydrates and lipids), as we could not be certain that all birds were post-absorptive before being placed in the chambers. EHL (W) was calculated from TEWL using a latent heat of vaporisation of $2.4 \text{ J mg}^{-1} \text{ H}_2\text{O}$ (corresponding with $T_a=40^\circ\text{C}$; Withers, 1992), and the efficiency of evaporative cooling was calculated as the ratio EHL/MHP.

All values are presented as means \pm s.d. Linear models (LM) and linear mixed-effects models (LME; *nlme* package; Pinheiro et al., 2009) were fitted to data using R 3.1.1 (R Development Core Team, 2014). The assumptions of all models (including normality, homogeneity of variance and multicollinearity), as well as model fit (residuals, leverage and Cook's *D*-values), were checked using the appropriate tests described in Logan (2010). An initial LM was fitted to M_b data, with site, season and sex as predictor variables, and as significant M_b variation was found, we included M_b in further analyses on physiological variables.

Little is known about the physiological processes responsible for among- and within-species differences in avian heat tolerance, and for this reason each response variable (T_b , TEWL, RMR and EHL/MHP) was analysed separately. All models were initially run including a set of potential predictor variables (M_b , site, season and sex) and interactions among these variables, and models were refined by comparing second-order Akaike information criterion values (AICc, *MuMIn* package) to determine which combination of predictor variables and interactions produced models that best fitted the datasets tested. Sex was initially included as a predictor variable, but as response variables never varied significantly with sex (all $P>0.05$), and its removal either improved or did not affect model fit (i.e. decrease or no change in AICc values), it was excluded from the final models on physiological variables.

We could not calculate inflection points representing the T_{uc} of individuals on account of too few data points to fit a segmented linear regression model (only four points per individual at $30^\circ\text{C} \leq T_a \leq 38^\circ\text{C}$), and thus considered $T_a \approx 30^\circ\text{C}$ (actual $T_a=30.1 \pm 0.2^\circ\text{C}$; $N=10$ per site per season) to be representative of thermoneutrality, as Smit and McKechnie (2010) found this T_a to be within the thermoneutral zone (TNZ) of sparrow-weavers. LMs were fitted to T_b , TEWL, RMR and EHL/MHP data at $T_a \approx 30^\circ\text{C}$, as well as to $T_{a,HT}$ data. *Post hoc* tests of multiple comparisons of means (Tukey contrasts for linear models; *multcomp* package; Hothorn et al., 2008) were used to identify between which sites, or site \times season groups, significant differences occurred.

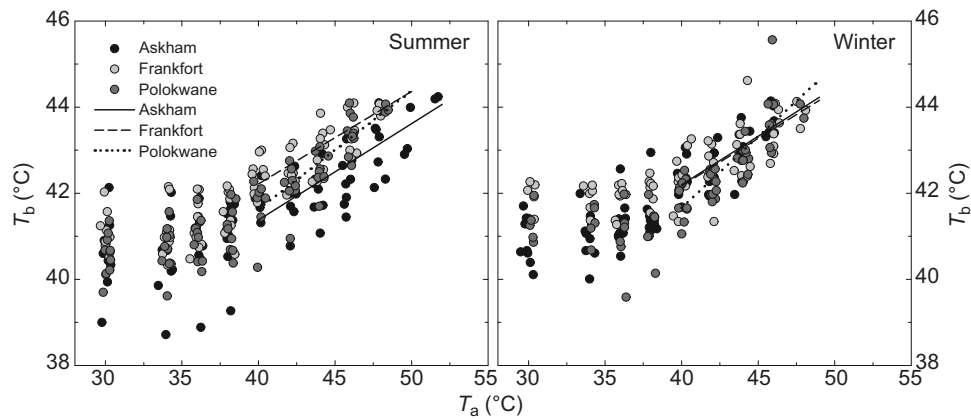


Fig. 1. Body temperature (T_b) of white-browed sparrow-weavers (*Plocepasser mahali*). Data were obtained during summer and winter at three sites in South Africa that vary in maximum summer air temperature ($T_{a,max}$) by $\sim 10^\circ\text{C}$. Different birds were exposed to $30^\circ\text{C} \leq T_a \leq 38^\circ\text{C}$ and $T_a \geq 40^\circ\text{C}$ ($N=10$ per site per season per T_a range), and T_b measured using passive integrated transponder tags. Lines indicate significant (all $P < 0.001$) linear mixed-effects regression models fitted to data at $T_a \geq 40^\circ\text{C}$. During summer, Askham: $y=0.22x+22.42$ ($t_{1,36}=11.556$), Frankfort: $y=0.22x+33.61$ ($t_{1,29}=10.907$) and Polokwane: $y=0.28x+30.58$ ($t_{1,24}=10.512$). During winter, Askham: $y=0.23x+33.15$ ($t_{1,22}=9.611$), Frankfort: $y=0.22x+33.33$ ($t_{1,30}=9.077$) and Polokwane: $y=0.33x+28.42$ ($t_{1,27}=11.627$).

LMEs were fitted to data (T_b , TEWL, RMR and EHL/MHP) at $T_a \geq 40^\circ\text{C}$ that included repeated measurements of birds at multiple T_a values ($N=10$ per site per season per T_a), and thus individual was included as a random effect. As T_b , TEWL and EHL/MHP varied significantly among site \times season groups, separate linear regression models were fitted within each group to investigate the respective relationships between T_a and the response variables, and analyses of covariance and *post hoc* tests were used to investigate how the slopes and y -intercepts of these regressions varied among site \times season groups. RMR did not vary among site \times season groups and thus separate linear regression models could not be fitted within each group. Moreover, we could not fit *post hoc* tests to investigate RMR and EHL/MHP variation among sites at $T_a \geq 40^\circ\text{C}$, because of the significant $T_a \times$ site and $T_a \times$ site \times season interactions, respectively, and thus fitted LMs to examine how RMR and EHL/MHP at $T_a \approx 42^\circ\text{C}$ (actual $T_a = 42.1 \pm 0.2^\circ\text{C}$; $N=10$ per site per season) varied with predictor variables, as this was the highest T_a that was below the $T_{a,HT}$ of all individuals (i.e. the highest T_a all birds reached without becoming hyperthermic).

RESULTS

Body mass

The M_b of sparrow-weavers varied significantly with site, but not between seasons, nor with the site \times season interaction (Table 2). The M_b of birds at all three sites differed significantly from each other ($P < 0.05$): birds at Frankfort were the largest (46.3 ± 3.7 g, $N=96$), followed by those at Polokwane (42.0 ± 4.0 g, $N=81$), and birds at Askham were the smallest (40.2 ± 3.7 g, $N=87$). The M_b of males (43.6 ± 4.7 g, $N=164$) was significantly greater than that of females (41.8 ± 4.3 g, $N=100$) across all sites and seasons (LM, $F_{2,257}=18.648$, $P < 0.001$).

Body temperature

The T_b of sparrow-weavers remained relatively stable at $T_a < 40^\circ\text{C}$, above which it increased linearly with increasing T_a (Fig. 1). The T_b of birds in their TNZ (actual $T_a = 30.1 \pm 0.2^\circ\text{C}$) was significantly lower ($\sim 0.7^\circ\text{C}$) in summer than in winter, but did not vary significantly with M_b among sites or with the site \times season interaction (Tables 2 and 3A). At $T_a \geq 40^\circ\text{C}$, T_b increased significantly with increasing T_a , and varied significantly with site, season and the site \times season interaction, but not with M_b (Table 2,

Fig. 1). Separate linear regressions of $T_a \geq 40^\circ\text{C}$ and T_b were fitted within each site \times season group, and T_b increased significantly with T_a in all groups (Fig. 1).

The slope of increasing T_b with $T_a \geq 40^\circ\text{C}$ was significantly steeper in Polokwane birds during both seasons than for the Askham and Frankfort populations (LME, $F_{5,221}=2.611$, $P=0.025$; Fig. 1). Furthermore, Polokwane birds did not show significant seasonal variation in their T_b response to increasing $T_a \geq 40^\circ\text{C}$ (slopes of increasing T_b with T_a : LME, $F_{1,69}=0.358$, $P=0.552$; y -intercepts: LME, $F_{1,70}=2.611$, $P=0.082$; Fig. 1, Table 4). The slope of increasing T_b with increasing $T_a \geq 40^\circ\text{C}$ in Askham and Frankfort birds did not vary significantly with the site \times season interaction (LME, $F_{3,152}=0.335$, $P=0.800$), but the y -intercepts did vary significantly with site \times season (LME, $F_{3,155}=21.610$, $P < 0.001$; Fig. 1, Table 4). This is reflected in variation of T_b values at a given $T_a \geq 40^\circ\text{C}$, and Askham birds maintained significantly lower T_b during summer than in winter (e.g. T_b was $\sim 0.7^\circ\text{C}$ lower in summer at $T_a \approx 42^\circ\text{C}$; Fig. 1, Table 4). Moreover, during summer, T_b at a given $T_a \geq 40^\circ\text{C}$ was significantly lower in the Askham population than in Frankfort birds (e.g. T_b was $\sim 0.7^\circ\text{C}$ lower at Askham at $T_a \approx 42^\circ\text{C}$; Fig. 1, Table 4).

In summary, T_b within the TNZ did not vary significantly among populations (Table 3A), but at $T_a \geq 40^\circ\text{C}$ several patterns of variation emerged (Fig. 1). Polokwane sparrow-weavers had significantly steeper slopes of increasing T_b with $T_a \geq 40^\circ\text{C}$ than the other two populations (Fig. 1). Furthermore, Askham sparrow-weavers had significantly lower T_b in summer at $T_a \geq 40^\circ\text{C}$ than in winter, and also had lower T_b values than Frankfort birds during summer (Fig. 1, Table 4).

Evaporative water loss rates

Rates of TEWL in all three populations remained relatively stable at lower T_a ($30\text{--}34^\circ\text{C}$), but increased linearly with higher T_a above an inflection point (Fig. 2). We could not calculate this inflection point (as explained in the data analysis section), but our limited behavioural observations indicated that birds started panting at $T_a = 36.8 \pm 2.7^\circ\text{C}$. At $T_a \approx 30^\circ\text{C}$ (in the TNZ), TEWL varied significantly with site and the site \times season interaction (Table 3B), but not with season or M_b (Table 2). There was no significant seasonal variation in TEWL in the TNZ within the three populations (Table 3B). However, during both seasons, the TEWL in the TNZ of

Table 2. Statistical results [F-value, P-value, degrees of freedom (d.f.) and sample size (n)] from linear models fitted to response variables of white-browed sparrow-weavers (*Plocepasser mahali*)

	T_a (°C)	$T_{a,max}$			M_b			Site			Season			Site×season		
		F-value	d.f., N	P-value	F-value	d.f., N	P-value	F-value	d.f., N	P-value	F-value	d.f., N	P-value	F-value	d.f., N	P-value
M_b	~30	–	–	–	–	–	–	71.638	2,259	<0.001	3.120	1,259	0.080	1.774	2,257	0.172
T_b	≥40	–	–	–	0.040	1,43	0.842	2.529	2,43	0.092	11.475	1,43	0.002	1.256	2,41	0.296
TEWL	~30	–	1,172	<0.001	1.700	1,172	0.193	4.800	2,53	0.012	8.900	1,53	0.004	1.700	2,53	0.005
	≥40	–	–	–	0.535	1,51	0.036	3.551	2,51	0.468	0.042	1,51	0.839	3.551	2,51	0.036
RMR	~30	–	1,192	<0.001	8.382	1,192	0.004	63.617	2,54	<0.001	13.031	1,54	<0.001	18.744	2,54	<0.001
	≥40	–	–	–	0.277	1,53	0.601	47.716	2,53	<0.001	8.262	1,53	<0.001	1.191	2,51	0.511
EHL/MHP	~30	–	1,190	0.004	5.809	1,190	0.017	82.167	2,56	<0.001	16.728	1,56	<0.001	1.807	2,54	0.174
	≥40	–	–	–	6.255	1,55	0.015	72.220	2,55	<0.001	11.502	1,55	0.005	1.211	2,53	0.306
$T_{a,HT}$	~30	–	–	–	7.781	1,52	0.007	8.717	2,52	<0.001	8.101	1,52	0.006	1.686	2,50	0.196
	≥40	–	1,187	<0.001	0.019	1,187	0.890	46.435	2,55	<0.001	80.628	1,55	<0.001	25.313	2,55	<0.001
	~42	–	–	–	0.068	1,53	0.796	21.448	2,53	<0.001	38.001	1,53	<0.001	6.503	2,53	0.003
	–	–	–	–	0.033	1,51	0.089	3.771	2,51	0.030	10.998	1,51	0.002	6.502	2,53	0.003

Data were collected during summer and winter at three sites in South Africa that vary in $T_{a,max}$ by ~10°C ($N=10$ per site per season). Birds were exposed to various T_a within a laboratory setup: at $T_a \approx 30^\circ\text{C}$ (in their thermoneutral zone; linear models), $T_a \geq 40^\circ\text{C}$ (linear mixed-effects models) and $T_a \approx 42^\circ\text{C}$ (linear models). N , sample size.

Response variables include: body mass (M_b), body temperature (T_b), total evaporative water loss (TEWL), resting metabolic rate (RMR), ratio of evaporative heat loss to metabolic heat production (EHL/MHP) and hyperthermia threshold air temperature ($T_{a,HT}$; the T_a at which $T_{b,HT} \approx 44^\circ\text{C}$). RMR and TEWL were measured using open flow-through respirometry, and T_b using passive integrated transponder tags. Predictor variables include: M_b (except when M_b was a response variable), site, season and the site×season interaction, as well as T_a for models at $T_a \geq 40^\circ\text{C}$. When a response variable varied significantly with a predictor variable, the P-value is presented in bold.

sparrow-weavers was significantly higher at Polokwane than at Askham (~167% and 100% higher in summer and winter, respectively) and Frankfort (~160% and 46% higher in summer and winter, respectively; Table 3B).

Rates of TEWL at $T_a \geq 40^\circ\text{C}$ increased significantly with increasing T_a and M_b , and also varied significantly with site, season and the site×season interaction (Table 2, Fig. 2). Separate linear regressions of TEWL versus $T_a \geq 40^\circ\text{C}$ were fitted within each site×season group, and TEWL increased significantly with T_a in all groups (Fig. 2). The slopes of these regressions did not significantly vary with the site×season interaction (LME, $F_{1,242}=0.816$, $P=0.540$), but the y-intercepts did significantly vary with site×season (LME, $F_{1,247}=104.400$, $P<0.001$), reflecting variation in TEWL at a given T_a value $\geq 40^\circ\text{C}$ (Fig. 2, Table 4). For example, at $T_a \approx 42^\circ\text{C}$ (actual $T_a=42.1 \pm 0.2^\circ\text{C}$), Polokwane and Frankfort sparrow-weavers had higher TEWL in summer than in winter (~28% and 20% higher, respectively), whereas the Askham population had lower TEWL in summer than in winter (~25% lower; Table 4).

In summary, TEWL in the TNZ of birds did not significantly differ between seasons, but did at $T_a \geq 40^\circ\text{C}$ (Fig. 2, Table 2). At both $T_a \approx 30^\circ\text{C}$ and $T_a \geq 40^\circ\text{C}$, Polokwane birds had significantly higher TEWL than the other two populations, regardless of season (Fig. 2, Tables 3B, 4). The same general patterns of significant TEWL variation were observed when analyses were repeated using mass-specific values.

Resting metabolic rate

The relationship between whole-animal RMR and T_a was less clear than those between T_a and other physiological variables (Fig. 3). In the TNZ of sparrow-weavers ($T_a \approx 30^\circ\text{C}$), RMR was significantly lower in summer than in winter (~22% lower), and also varied significantly among sites, but not with the site×season interaction or M_b (Table 2). Polokwane sparrow-weavers had significantly higher RMR in their TNZ than both Frankfort and Askham birds (~93% and 202% higher, respectively; Table 3A).

At $T_a \geq 40^\circ\text{C}$, RMR was significantly lower in summer than in winter, increased significantly with increasing T_a and M_b (Table 2), and varied significantly with site (Table 2) and the $T_a \times$ site interaction (LME, $F_{1,54}=18.744$, $P=0.008$). However, RMR did not vary significantly with the site×season interaction (Table 2), so we could not fit separate regression models within each site×season group (hence the absence of regression lines in Fig. 3). Moreover, *post hoc* tests to investigate variation among sites could not be fitted because of the significant $T_a \times$ site interaction; thus, RMR values at $T_a \approx 42^\circ\text{C}$ were analysed instead. At $T_a \approx 42^\circ\text{C}$, RMR was also significantly lower in summer than in winter (~14% lower), and varied significantly with M_b and site, but not with the site×season interaction (Table 2). The Polokwane population had a significantly higher RMR at $T_a \approx 42^\circ\text{C}$ compared with both Frankfort and Askham birds (~53% and 96% higher, respectively; Table 3A).

In summary, RMR in the TNZ of birds, at $T_a \geq 40^\circ\text{C}$ and at $T_a \approx 42^\circ\text{C}$ was significantly higher in winter than in summer (Fig. 3, Table 2). Furthermore, RMR at both $T_a \approx 30^\circ\text{C}$ and $T_a \approx 42^\circ\text{C}$ was significantly higher in the Polokwane population than in Askham and Frankfort sparrow-weavers (Table 3A). The same general patterns of significant RMR variation were observed when analyses were repeated using mass-specific values.

Ratio of evaporative heat loss to metabolic heat production

The EHL/MHP ratio remained relatively stable at lower T_a (30–34°C), but increased linearly with higher T_a above an inflection point (Fig. 4). During winter, EHL/MHP of the Polokwane and Frankfort

Table 3. Body temperature (T_b), total evaporative water loss (TEWL), resting metabolic rate (RMR), ratio of evaporative heat loss to metabolic heat production (EHL/MHP) and hyperthermia threshold air temperature ($T_{a,HT}$) of white-browed sparrow-weavers (*Plocepasser mahali*)

(A)	Season		Site			
	Summer	Winter	Askham	Frankfort	Polokwane	
T_b (°C) at $T_a \approx 30^\circ\text{C}$	40.7±0.2	41.4±0.5	40.8±0.2	41.4±0.7	40.9±0.5	
RMR (W) at $T_a \approx 30^\circ\text{C}$	0.78±0.43	1.00±0.59	0.48±0.12	0.75±0.22	1.45±0.51	
RMR (W) at $T_a \approx 42^\circ\text{C}$	0.73±0.41	0.85±0.28	0.56±0.15	0.72±0.10	1.10±0.22	
EHL/MHP at $T_a \approx 30^\circ\text{C}$	0.26±0.05	0.22±0.06	0.28±0.04	0.22±0.05	0.22±0.06	
Site×season group						
(B)	Askham		Frankfort		Polokwane	
	Summer	Winter	Summer	Winter	Summer	Winter
Daily T_{max} (°C)	37.7±3.7	23.8±2.8	29.3±2.5	19.7±2.3	26.9±2.1	21.2±3.5
TEWL (mg min ⁻¹) at $T_a \approx 30^\circ\text{C}$	3.13±1.03	3.48±0.53	3.20±0.57	4.77±0.98	8.27±2.14	7.02±1.82
EHL/MHP at $T_a \approx 42^\circ\text{C}$	1.39±0.26	1.30±0.08	1.25±0.12	0.94±0.12	1.32±0.17	0.89±0.06
$T_{a,HT}$ (°C)	49.5±1.4	46.8±0.9	47.3±1.5	47.3±1.5	47.6±1.3	46.7±1.2

Measurements were taken during summer and winter at three sites in South Africa that differ in maximum summer T_a ($T_{a,max}$) by $\sim 10^\circ\text{C}$. RMR and TEWL were measured using open flow-through respirometry and T_b using passive integrated transponder tags, at various temperatures including: $T_a \approx 30^\circ\text{C}$ and $T_a \approx 42^\circ\text{C}$ ($n=10$ per site per season per T_a ; mean±s.d.). T_b , RMR and EHL/MHP at $T_a \approx 30^\circ\text{C}$, as well as RMR at $T_a \approx 42^\circ\text{C}$, did not vary with the site×season interaction, and thus these values are presented for each site and season separately (A). However, TEWL at $T_a \approx 30^\circ\text{C}$, EHL/MHP at $T_a \approx 42^\circ\text{C}$ and $T_{a,HT}$ (air temperature at which $T_b \approx 44^\circ\text{C}$) varied with the site×season interaction, and thus values are presented for each site×season group (B).

Significance values (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) of the differences among site and season categories (relationships shown by dashed lines) are from linear models and *post hoc* tests of multiple comparisons of means (Tukey contrasts).

population reached a plateau at higher T_a (~ 44 and 46°C , respectively), suggesting maximum evaporative capacity for heat dissipation had been reached (Fig. 4). In the TNZ of sparrow-weavers ($T_a \approx 30^\circ\text{C}$), EHL/MHP was significantly higher in summer than in winter, and also varied significantly among sites and M_b , but not with the site×season interaction (Table 2). Askham sparrow-weavers had significantly higher EHL/MHP at $T_a \approx 30^\circ\text{C}$ than both Frankfort and Polokwane birds (Table 3A).

At $T_a \geq 40^\circ\text{C}$, EHL/MHP increased significantly with increasing T_a , and varied significantly with site, season, the site×season interaction and the T_a ×site×season interaction (LME, $F_{1,187}=11.855$, $P<0.001$; Fig. 4), but not with M_b (Table 2). *Post hoc* tests to investigate variation among site×season groups could not be fitted because of the significant T_a ×site×season interaction, thus RMR values at $T_a \approx 42^\circ\text{C}$ were analysed instead. At $T_a \approx 42^\circ\text{C}$, EHL/MHP varied significantly with site, season and the site×season interaction, but not with M_b (Table 2). Polokwane and Frankfort sparrow-weavers had higher EHL/MHP at $T_a \approx 42^\circ\text{C}$ in summer than in winter ($\sim 49\%$ and 21% higher, respectively), whereas no significant seasonal variation occurred in the Askham population

(Table 3B). During summer, no variation in EHL/MHP occurred among the three populations, but during winter, Askham birds had higher EHL/MHP than both Polokwane and Frankfort sparrow-weavers (Table 3B).

In summary, EHL/MHP ratios in the TNZ were significantly higher in Askham birds than in the other two populations (Fig. 4, Table 3A). At $T_a \approx 42^\circ\text{C}$, seasonal variation of EHL/MHP occurred within the Polokwane and Frankfort populations, but not in Askham sparrow-weavers, and the latter population therefore had higher EHL/MHP at $T_a \approx 42^\circ\text{C}$ than birds from the other two sites during winter (Fig. 4, Table 3B).

Hyperthermia threshold air temperature

The $T_{a,HT}$ of sparrow-weavers (i.e. T_a at which $T_b \approx 44^\circ\text{C}$; actual $T_b = 44.3^\circ\text{C}$ after calibration) varied significantly among seasons and sites, and with the site×season interaction, but not with M_b (Table 2). Askham sparrow-weavers had a significantly higher $T_{a,HT}$ ($\sim 2.7^\circ\text{C}$ higher) in summer than in winter, but there was no significant seasonal variation in $T_{a,HT}$ within the other two populations (Table 3B). During summer, sparrow-weavers at

Table 4. Body temperature (T_b) and total evaporative water loss (TEWL) of white-browed sparrow-weavers (*Plocepasser mahali*) significantly increase with $T_a \geq 40^\circ\text{C}$

	Askham		Frankfort		Polokwane	
	Summer	Winter	Summer	Winter	Summer	Winter
Daily $T_{a,max}$ ($^\circ\text{C}$)	37.7 \pm 3.7	23.8 \pm 2.8	29.3 \pm 2.5	19.7 \pm 2.3	26.9 \pm 2.1	21.2 \pm 3.5
T_b at $T_a \approx 42^\circ\text{C}$ ($^\circ\text{C}$)	41.8 \pm 0.5	42.5 \pm 0.5	42.5 \pm 0.4	42.5 \pm 0.6	41.7 \pm 0.7 ^a	42.2 \pm 0.3 ^a
y -intercepts of regressions at $T_a \geq 40^\circ\text{C}$						
TEWL (mg min^{-1}) at $T_a \approx 42^\circ\text{C}$	15.8 \pm 2.1	21.2 \pm 3.3	20.4 \pm 3.5	17.0 \pm 1.7	33.3 \pm 4.5	26.0 \pm 5.9
y -intercepts of regressions at $T_a \geq 40^\circ\text{C}$						

Measurements were taken during summer and winter at three sites in South Africa that differ in $T_{a,max}$ by $\sim 10^\circ\text{C}$. The y -intercepts of linear mixed-effects regressions varied significantly, reflecting variation in response variables at a given $T_a \geq 40^\circ\text{C}$ among site \times season groups. We therefore present T_b and TEWL values at $T_a \approx 42^\circ\text{C}$ (means \pm s.d.) along with the results from *post hoc* tests of multiple comparisons of means (Tukey contrasts) on the y -intercepts ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$; relationships shown by dashed lines). The y -intercepts of Polokwane regressions at $T_a \geq 40^\circ\text{C}$ could not be compared with those of Askham and Frankfort, as the slopes of the Polokwane regressions significantly differed from the other two sites. TEWL was measured using open flow-through respirometry, and T_b using passive integrated transponder tags.

Askham exhibited significantly higher $T_{a,HT}$ values than conspecifics at Polokwane and Frankfort (~ 1.9 and 2.2°C higher, respectively); however, $T_{a,HT}$ did not vary significantly among the three populations during winter (Table 3B).

DISCUSSION

We found significant intraspecific seasonal and spatial variation in the evaporative cooling capacity and heat tolerance of *P. mahali*, manifested as significant differences in the maximum T_a birds were able to tolerate before the onset of severe hyperthermia. As predicted, sparrow-weavers at the hot desert site (Askham) with the greatest seasonal variation in $T_{a,max}$ values tolerated significantly higher T_a during summer than conspecifics at cooler sites, and were the only population to show significant seasonal acclimatisation in $T_{a,HT}$ (Table 3B). Askham sparrow-weavers demonstrated thermoregulatory responses broadly similar to those reported for this species by Whitfield et al. (2015) at two climatically similar sites, Wildsgenot Game Ranch ($27^\circ 04'S$, $21^\circ 23'E$) and Leeupan Ranch ($26^\circ 58'S$, $21^\circ 50'E$).

Thermoneutral T_b values were indistinguishable among sparrow-weaver populations, as was the case for populations of *E. alpestris*

(Trost, 1972) and *Passer domesticus* (Hudson and Kimzey, 1966). However, significant T_b variation among populations emerged at $T_a \geq 40^\circ\text{C}$, with Polokwane sparrow-weavers increasing T_b with increasing $T_a \geq 40^\circ\text{C}$ at a faster rate than in the Askham and Frankfort populations. The latter two populations had slopes of increasing T_b with increasing $T_a \geq 40^\circ\text{C}$ similar to those previously reported for Kalahari sparrow-weavers during summer at $40^\circ\text{C} < T_a < 48^\circ\text{C}$ (Whitfield et al., 2015).

The seasonal acclimatisation in T_b at $T_a \geq 40^\circ\text{C}$ in desert sparrow-weavers is a novel finding, with the only other study of seasonality of heat tolerance at high T_a of which we are aware finding no significant acclimatisation in captive-reared *C. macqueenii* (Tieleman et al., 2002b). However, the validity of this comparison is questionable as *C. macqueenii* (1200 g) is a substantially larger bird than *P. mahali*, and it is also unclear whether captivity could have had an impact on the physiological responses of these birds (Tieleman et al., 2002b). The reduction of T_b during summer at $T_a \geq 40^\circ\text{C}$ that was observed in Askham sparrow-weavers resulted in this population also having significantly lower T_b than conspecifics from a more mesic population in summer (Frankfort; $\sim 0.7^\circ\text{C}$ difference in T_b at $T_a = 42^\circ\text{C}$; Table 4). This pattern of among-

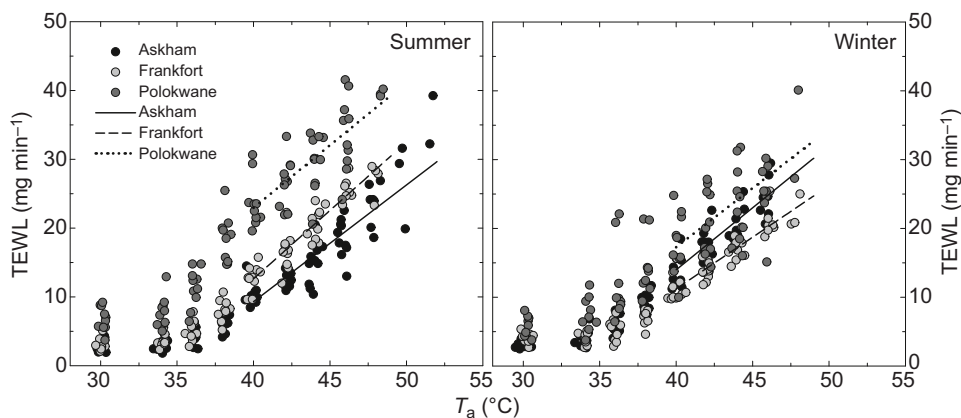


Fig. 2. Total evaporative water loss (TEWL) rates of white-browed sparrow-weavers (*Plocepasser mahali*). Data were obtained during summer and winter at three sites in South Africa that vary in $T_{a,max}$ by $\sim 10^\circ\text{C}$. Different birds were measured at $30^\circ\text{C} \leq T_a \leq 38^\circ\text{C}$ and $T_a \geq 40^\circ\text{C}$ ($N=10$ per site per season per T_a range), and TEWL measured using open flow-through respirometry. Lines indicate significant (all $P < 0.001$) linear mixed-effects regression models fitted to data at $T_a \geq 40^\circ\text{C}$. During summer, Askham: $y=1.70x-58.54$ ($t_{1,41}=16.967$), Frankfort: $y=1.99x-66.77$ ($t_{1,30}=17.618$) and Polokwane: $y=1.82x-49.74$ ($t_{1,32}=11.763$). During winter, Askham: $y=1.80x-58.51$ ($t_{1,26}=13.615$), Frankfort: $y=1.50x-48.78$ ($t_{1,30}=16.505$) and Polokwane: $y=1.72x-51.45$ ($t_{1,26}=10.885$).

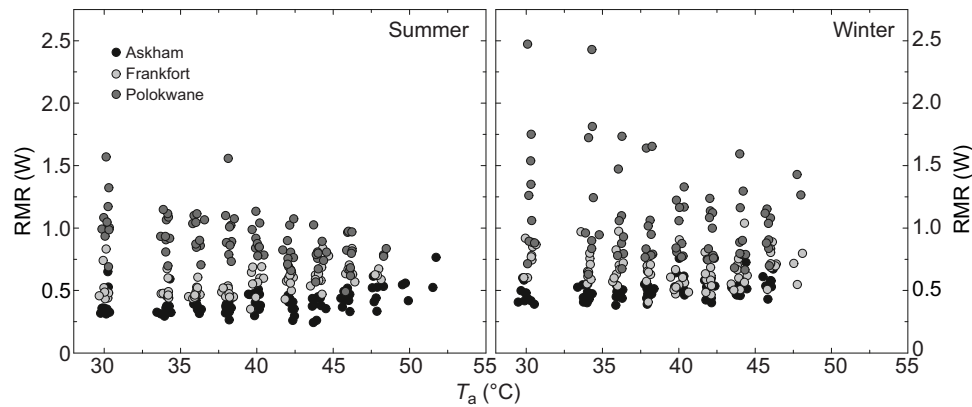


Fig. 3. Resting metabolic rate (RMR) of white-browed sparrow-weavers (*Plocepasser mahali*). Data were obtained during summer and winter at three sites in South Africa that vary in $T_{a,max}$ by $\sim 10^{\circ}\text{C}$. Different birds were measured at $30^{\circ}\text{C} \leq T_a \leq 38^{\circ}\text{C}$ and $T_a \geq 40^{\circ}\text{C}$ ($N=10$ per site per season per T_a range), and RMR measured using open flow-through respirometry. RMR increased significantly with increasing $T_a \geq 40^{\circ}\text{C}$ and also varied significantly among sites and seasons. As RMR did not significantly vary among site \times season groups and the $T_a \times$ site interaction was significant, we did not fit separate regression models or *post hoc* tests to investigate variation among sites at $T_a \geq 40^{\circ}\text{C}$, and thus RMR values at $T_a \approx 42^{\circ}\text{C}$ were analysed instead.

population variation in T_b is similar to that observed in desert and mesic populations of *E. alpestris* at $T_a=45^{\circ}\text{C}$ ($\sim 1.7^{\circ}\text{C}$ difference in T_b ; Trost, 1972). Moreover, these patterns are consistent with our predictions, as sparrow-weavers from the hot desert site increased T_b more slowly with increasing $T_a \geq 40^{\circ}\text{C}$ compared with conspecifics from a mesic site (Polokwane), and maintained lower T_b at a given $T_a \geq 40^{\circ}\text{C}$ compared with birds from the other mesic site (Frankfort).

Facultative hyperthermia is thought to contribute to the ability of birds to survive in hot environments by decreasing the thermal gradient between their surface and the environment, thereby conserving water by reducing EWL (Dawson, 1958; Trost, 1972; Weathers, 1981). Nord and Williams (2015), for instance, estimated that incubating greater hoopoe larks (*Alaemon alaudipes*) can reduce TEWL by 15–20% at a T_a of 40°C by increasing T_b from 42 to 45°C . However, the notion that the capacity for facultative hyperthermia may be greater in desert birds than in mesic species is not supported by the finding that the magnitude of hyperthermic responses did not differ between desert and non-desert birds at $T_b=45^{\circ}\text{C}$ (Tieleman and Williams, 1999). It is also possible that maintaining a lower T_b has an adaptive value by providing a greater

capacity for heat storage before lethal T_b limits are reached (McNab and Morrison, 1963; Tieleman et al., 2002a).

Smit et al. (2013) found that in two free-ranging populations of *P. mahali* in the Kalahari Desert, the T_b set-point was significantly higher in a desert ($41.5 \pm 0.2^{\circ}\text{C}$) versus semi-desert population ($40.2 \pm 0.2^{\circ}\text{C}$), but that the desert population did not have a greater capacity for hyperthermia (i.e. $T_b >$ modal T_b values). In contrast to these free-ranging populations, our laboratory data for *P. mahali* (present study) and those of Trost (1972) on *E. alpestris* reveal lower T_b in desert than in mesic populations at high T_a . Moreover, free-ranging sparrow-weavers commenced panting at $T_a > 28^{\circ}\text{C}$ (Smit et al., 2013), whereas our limited behavioural observations indicated that birds in the laboratory started panting at a substantially higher T_a across all sites (mean T_a at onset of panting = $36.8 \pm 2.7^{\circ}\text{C}$; lowest $T_a = 32.0^{\circ}\text{C}$). These differences likely reflect the very low chamber humidities in the present study and the effects of solar radiation on the operative temperatures experienced by free-ranging sparrow-weavers. Differences in physiological responses to acute heat stress between natural habitats and artificial conditions largely preclude the extrapolation of laboratory data to free-ranging birds; however, the goal of the

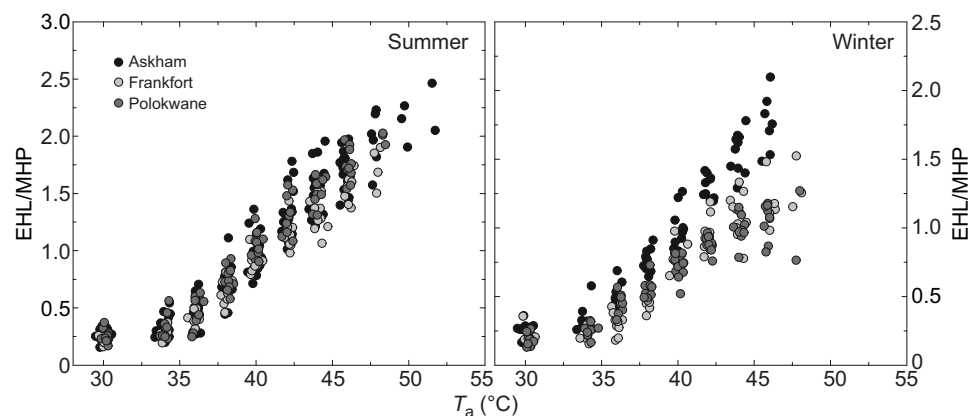


Fig. 4. The ratio of evaporative heat loss to metabolic heat production (EHL/MHP) of white-browed sparrow-weavers (*Plocepasser mahali*). Data were obtained during summer and winter at three sites in South Africa that vary in $T_{a,max}$ by $\sim 10^{\circ}\text{C}$. Different birds were measured at $30^{\circ}\text{C} \leq T_a \leq 38^{\circ}\text{C}$ and $T_a \geq 40^{\circ}\text{C}$ ($N=10$ per site per season per T_a range), and metabolic variables measured using open flow-through respirometry. EHL/MHP increased significantly with increasing $T_a \geq 40^{\circ}\text{C}$ and also varied significantly among sites and seasons. Because of the significant $T_a \times$ site \times season interaction, we did not fit separate regression models or *post hoc* tests to investigate variation among site \times season groups at $T_a \geq 40^{\circ}\text{C}$, and thus EHL/MHP values at $T_a \approx 42^{\circ}\text{C}$ were analysed instead.

present study was to quantify intraspecific variation in physiological responses to high T_a in a manner allowing for direct comparisons among populations.

At $T_a \approx 30^\circ\text{C}$ and $T_a \geq 40^\circ\text{C}$, TEWL in the desert population (Askham) was similar to that reported by Whitfield et al. (2015) for Kalahari sparrow-weavers during summer ($\sim 2.69 \text{ mg min}^{-1}$ at $25^\circ\text{C} < T_a < 35^\circ\text{C}$ and $\sim 11.71 \text{ mg min}^{-1}$ at $T_a = 42^\circ\text{C}$). The reduction in both TEWL and T_b during summer compared with winter in desert sparrow-weavers suggests that these birds can enhance evaporative cooling to conserve water and cope with high summer $T_{a,\text{max}}$ (Table 1). Seasonal adjustments in TEWL have also been demonstrated in *C. macqueenii*, with reduced summer TEWL at $T_a = 35^\circ\text{C}$ ($\sim 32\%$ lower compared with winter values), but not at $T_a = 50^\circ\text{C}$ (Tieleman et al., 2002b).

Many arid-zone birds have been found to have lower TEWL at thermoneutrality compared with mesic-zone species (Tieleman et al., 2002a, 2003a; Williams, 1996), and some studies suggest that similar variation may exist at the intraspecific level (MacMillen and Hinds, 1998; Sabat et al., 2006). Our results provide only limited support for this idea, as TEWL in the TNZ was significantly higher in a mesic population (Polokwane) compared with both the desert (Askham) and the other mesic population (Frankfort; Table 3B). However, at $T_a \geq 40^\circ\text{C}$, variation in TEWL was consistent with our predictions, with the desert population having significantly lower TEWL than either mesic population – a difference similar to that observed between desert and mesic populations of *E. alpestris* ($\sim 32\%$ lower in the desert population at $T_a = 45^\circ\text{C}$; Trost, 1972). The adaptive value of lower TEWL in desert birds is thought to concern water conservation (Tieleman et al., 2002a; Williams, 1996; Williams and Tieleman, 2000), and our results suggest that this pattern may become more pronounced with increasing $T_a > T_b$ (Dawson and Whittow, 2000; Williams, 1999).

The lack of an obvious increase in RMR with increasing T_a , despite the obvious increase of T_b and TEWL, is puzzling. However, this relationship between RMR and T_a is consistent with previous work at similar T_a ranges on birds in the Kalahari desert, including *P. mahali* and two other ploceid passerines (Whitfield et al., 2015), and three columbids (*Oena capensis*, *Spilopelia senegalensis* and *Streptopelia capicola*; M. C. Whitfield, B. Smit, A.E.M. and B.O. W., unpublished data). The RMR of the Askham population was similar to that previously observed in Kalahari sparrow-weavers during summer (Whitfield et al., 2015). A number of studies have demonstrated lower metabolic rates in desert compared with mesic birds at moderate temperatures (Sabat et al., 2006; Tieleman et al., 2002a, 2003a; Tieleman and Williams, 2000), but we could only find one study demonstrating lower RMR at $T_a > T_b$ in a desert compared with a mesic population ($\sim 32\%$ lower at $T_a = 45^\circ\text{C}$ in *E. alpestris*; Trost, 1972). In contrast to our predictions, there was no clear pattern of RMR variation among desert versus mesic populations in the present study; corresponding variation in BMR is also absent in *P. mahali* (M.J.N., B.O.W. and A.E.M., unpublished data).

Sparrow-weavers in the present study had EHL/MHP ratios ranging from ~ 0.20 at thermoneutrality to maximum values of ~ 1.00 – 2.31 . These maximum EHL/MHP ratios are similar to the value reported previously for Kalahari sparrow-weavers during summer (1.93; Whitfield et al., 2015) and for other avian species (Lasiewski et al., 1966; Lasiewski and Seymour, 1972; Trost, 1972). The lack of seasonal variation in EHL/MHP ratios at $T_a \approx 42^\circ\text{C}$ in desert (Askham) sparrow-weavers contrasts with the reduction in both TEWL and T_b during summer in this population.

The mechanisms allowing birds to adjust TEWL as a component of seasonal acclimatisation have received less attention than those

underlying seasonal adjustments in metabolic variables such as basal and summit metabolism. Several mechanisms have been proposed as drivers of lower TEWL in arid-zone birds compared with their mesic counterparts, including an increased capacity for facultative hyperthermia (Dawson, 1958; Trost, 1972; Weathers, 1981), countercurrent heat exchange in the nasal passages (Geist, 2000; Schmidt-Nielsen et al., 1970) and a reduction in cutaneous evaporative water loss (CEWL) by adjustments in the lipid composition of the epidermis (Menon et al., 1989; Tieleman and Williams, 2002; Webster and Bernstein, 1987; Williams, 1996). As discussed above, lower summer T_b in desert sparrow-weavers implies that facultative hyperthermia is not the mechanism responsible for reduced summer TEWL in this population during acute heat exposure. Furthermore, seasonal variation in TEWL was only significant at $T_a \geq 40^\circ\text{C}$, suggesting this is not the result of countercurrent heat exchange in the nasal passages or of reduced CEWL; both of these mechanisms should be more efficient at moderate T_a , as passerines typically respond to increasing $T_a > T_b$ by an increase in respiratory evaporation via panting (see also Geist, 2000; Sabat et al., 2006; Schmidt-Nielsen et al., 1970; Tieleman et al., 1999; Tieleman and Williams, 2002). Alternately, adjustments in respiratory variables may enhance the evaporative efficiency of panting. Although adjustments in the rate of EWL are well studied (Dawson, 1982; Richards, 1970; Wolf and Walsberg, 1996), mechanisms that could potentially increase the energy dissipated evaporatively per unit energy expended on muscle contractions during panting remain unclear. One potential mechanism concerns changes in the elastic properties of avian respiratory systems to increase the resonant frequency of respiration during summer, thereby resulting in an increase in EHL without an associated change in MHP (Crawford and Kampe, 1971; Richards, 1970). However, such changes would be reflected as increased EHL/MHP in summer, whereas no such increases were evident in the Askham sparrow-weavers.

Patterns of variation in the maximum T_a that sparrow-weavers could tolerate before the onset of severe hyperthermia (i.e. $T_{a,\text{HT}}$; $T_b \approx 44^\circ\text{C}$) were closely linked to the $T_{a,\text{max}}$ experienced by each population, with seasonal acclimatisation of $T_{a,\text{HT}}$ observed only in the desert population. The greater heat tolerance of Askham sparrow-weavers appears to result from enhanced evaporative cooling at $T_a \geq 40^\circ\text{C}$ during summer compared with winter, as at a given $T_a \geq 40^\circ\text{C}$, these birds lost less water by TEWL during summer than in winter, but could still maintain lower T_b values during summer, allowing them to conserve water and reach higher T_a values before the onset of severe hyperthermia. To ensure that the upper thermoregulatory limit was quantified, Whitfield et al. (2015) allowed birds in the Kalahari Desert to reach substantially higher T_b (maximum $T_b = 45.5 \pm 0.1^\circ\text{C}$) than in the present study, and as a result the maximum T_a values in their study were as high as $T_a \approx 54^\circ\text{C}$. However, the aim of the present study was to use a standardised protocol to compare the heat tolerance and evaporative cooling capacity of *P. mahali* populations between seasons and among populations, and as sparrow-weavers from the more mesic sites displayed clear signs of behavioural and physiological stress at $T_b > 44^\circ\text{C}$, we used this T_b value as a cut-off during all measurements. The $T_{a,\text{HT}}$ is a novel metric for comparative analyses, and moreover is probably the most ecologically significant variable in this study, as it quantifies the combined effect of the thermoregulatory variables (TEWL, RMR and T_b) that resulted in the up-regulation of summer heat tolerance and evaporative cooling capacity compared with winter in the desert population.

We quantified thermoregulatory variation among *P. mahali* populations along a climatic gradient, but our data do not permit us to infer the processes responsible for these phenotypic differences. The patterns of physiological variation we observed could arise from local adaptation, but might also reflect phenotypic plasticity through acclimatisation to current conditions or developmental plasticity to conditions experienced during development (Piersma and Drent, 2003; Pigliucci, 2001). Moreover, the small number of populations we examined also constrains our ability to make any inferences in this regard (Garland and Adolph, 1994; Hurlbert, 1984). The present study should instead be seen as an initial step towards identifying a model species suitable for experimental work designed to tease apart the roles of local adaptation versus phenotypic plasticity in determining these among-population differences, using common-garden and short-term thermal acclimation experiments. We argue that *P. mahali* is a suitable model for such studies, as intraspecific variation in thermoregulatory responses to high T_a have been demonstrated under both free-ranging (Smit et al., 2013) and laboratory conditions (present study).

Increasing evidence of fine-scale intraspecific physiological variation lends further support to the idea of adaptive thermoregulation and the thermal physiology of endotherms being more flexible than previously recognised (Angilletta et al., 2010; Glanville et al., 2012; Smit et al., 2013). A number of studies have demonstrated seasonal acclimatisation in avian basal and summit metabolism in relation to cold tolerance (reviewed by McKechnie and Swanson, 2010), but to the best of our knowledge this is the first study to reveal distinct seasonal acclimatisation in avian evaporative cooling capacity and heat tolerance. Pronounced seasonal adjustments in heat tolerance occurred in the population that experienced the most pronounced variation in climate (Table 1), which suggests that birds could have the potential to show adaptive physiological responses when faced with changing climates. Future studies are required to establish whether seasonal acclimatisation in heat tolerance and evaporative cooling capacity is widespread among birds inhabiting hot environments, and to investigate the evolutionary processes driving intraspecific variation in avian thermoregulatory responses to high T_a .

Acknowledgements

We thank the Rossouw family for allowing us to conduct research work on their property, the owners and managers of Frankfort River Resort and Polokwane Game Reserve for their assistance, and the South African Weather Service for providing climate data. We are grateful to Ben and Maxine Smit for advice, and Michelle Thompson, Ryan O'Connor, Mervyn Uys, Natasha Visser, Alexandra Howard and Rowan Jordaan for field assistance. We also thank Bruce Woodroffe and Awesome Tools (Cape Town, South Africa) for discounted lighting equipment. Berry Pinshow and an anonymous reviewer provided constructive comments on an earlier manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

A.E.M. and B.O.W. conceived and designed the study. M.J.N. performed the experiments and analysed the data with guidance from A.E.M. and B.O.W. M.J.N. and A.E.M. wrote the manuscript, and B.O.W. provided editorial comments and advice.

Funding

The study was facilitated by funding from the Department of Science and Technology-National Research Foundation (DST-NRF) Centre of Excellence at the Percy FitzPatrick Institute (to A.E.M.) and University of Pretoria (to A.E.M.), and the National Science Foundation [IOS-1122228 to B.O.W.]. Any opinions, findings and conclusions or recommendations expressed in this material are those

of the authors and do not necessarily reflect the views of the National Science Foundation.

References

- Ambrose, S. J., Bradshaw, S. D., Withers, P. C. and Murphy, D. P. (1996). Water and energy balance of captive and free-ranging spinifexbirds (*Eremiornis carteri*) North (Aves: Sylviidae) from Barrow Island, Western Australia. *Aust. J. Ornithol.* **44**, 107–117.
- Angilletta, M. J., Jr, Cooper, B. S., Schuler, M. S. and Boyles, J. G. (2010). The evolution of thermal physiology in endotherms. *Front. Biosci.* **E2**, 861–881.
- Boyles, J. G., Seebacher, F., Smit, B. and McKechnie, A. E. (2011). Adaptive thermoregulation in endotherms may alter responses to climate change. *Integr. Comp. Biol.* **51**, 676–690.
- Cade, T. J. (1965). Survival of the scaly-feathered finch *Sporopipes squamifrons* without drinking water. *Ostrich* **36**, 131–132.
- Calder, W. A. and Schmidt-Nielsen, K. (1967). Temperature regulation and evaporation in the pigeon and the roadrunner. *Am. J. Physiol.* **213**, 883–889.
- Cavieres, G. and Sabat, P. (2008). Geographic variation in the response to thermal acclimation in rufous-collared sparrows: are physiological flexibility and environmental heterogeneity correlated? *Funct. Ecol.* **22**, 509–515.
- Crawford, E. C. and Kampe, G. (1971). Resonant panting in pigeons. *Comp. Biochem. Physiol. A Physiol.* **40**, 549–552.
- Crawford, E. C. and Schmidt-Nielsen, K. (1967). Temperature regulation and evaporative cooling in the ostrich. *Am. J. Physiol.* **212**, 347–353.
- Dawson, W. R. (1958). Relation of oxygen consumption and evaporative water loss to temperature in the cardinal. *Physiol. Zool.* **31**, 37–48.
- Dawson, W. R. (1982). Evaporative losses of water by birds. *Comp. Biochem. Physiol. A Physiol.* **71**, 495–509.
- Dawson, W. R. and Bartholomew, G. A. (1968). Temperature regulation and water economy of desert birds. In *Desert Biology* (ed. G. W. Brown), pp. 357–394. New York: Academic press.
- Dawson, W. R. and Whittow, G. C. (2000). Regulation of body temperature. In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 343–390. New York: Academic Press.
- du Plessis, M. A. (2005). White-browed sparrow-weaver (*Plocepasser mahali*). In *Roberts Birds of Southern Africa* (ed. P. A. R. Hockey, W. R. J. Dean and P. G. Ryan), pp. 1006–1007. Cape Town: Trustees of the John Voelcker Bird Book Fund.
- du Plessis, K. L., Martin, R. O., Hockey, P. A. R., Cunningham, S. J. and Ridley, A. R. (2012). The costs of keeping cool in a warming world: implications of high temperatures for foraging, thermoregulation and body condition of an arid-zone bird. *Global Change Biol.* **18**, 3063–3070.
- Garland, T., Jr and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**, 797–828.
- Geist, N. R. (2000). Nasal respiratory turbinate function in birds. *Physiol. Biochem. Zool.* **73**, 581–589.
- Glanville, E. J., Murray, S. A. and Seebacher, F. (2012). Thermal adaptation in endotherms: climate and phylogeny interact to determine population-level responses in a wild rat. *Funct. Ecol.* **26**, 390–398.
- Hothorn, T., Bretz, F. and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical J.* **50**, 346–363.
- Hudson, J. W. and Kimzey, S. L. (1966). Temperature regulation and metabolic rhythms in populations of the house sparrow, *Passer domesticus*. *Comp. Biochem. Physiol.* **17**, 203–217.
- Hurlbert, S. H. (1984). Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54**, 187–211.
- Intergovernmental Panel on Climate Change (2012). *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation*. Cambridge; New York: Cambridge University Press.
- Lasiewski, R. C. and Seymour, R. S. (1972). Thermoregulatory responses to heat stress in four species of birds weighing approximately 40 grams. *Physiol. Zool.* **45**, 106–118.
- Lasiewski, R. C., Acosta, A. L. and Bernstein, M. H. (1966). Evaporative water loss in birds – I. Characteristics of the open flow method of determination, and their relation to estimates of thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445–457.
- Logan, M. (2010). *Biostatistical Design and Analysis Using R: A Practical Guide*. Chichester: Wiley-Blackwell.
- MacMillen, R. E. and Hinds, D. S. (1998). Water economy of granivorous birds: California house finches. *Condor* **100**, 493–503.
- McKechnie, A. E. (2008). Phenotypic flexibility in basal metabolic rate and the changing view of avian physiological diversity: a review. *J. Comp. Physiol. B* **178**, 235–247.
- McKechnie, A. E. and Swanson, D. L. (2010). Sources and significance of variation in basal, summit and maximal metabolic rates in birds. *Curr. Zool.* **56**, 741–758.
- McNab, B. K. and Morrison, P. (1963). Body temperature and metabolism in subspecies of *Peromyscus* from arid and mesic environments. *Ecol. Monogr.* **33**, 63–82.

- Menon, G. K., Baptista, L. F., Brown, B. E. and Elias, P. M.** (1989). Avian epidermal differentiation. II. Adaptive response of permeability barrier to water deprivation and replenishment. *Tissue Cell* **21**, 83–92.
- Nord, A. and Williams, J. B.** (2015). The energetics cost of incubation. In *Nests, Eggs and Incubation: New Ideas about Avian Reproduction* (ed. D. C. Deeming and S. J. Reynolds), pp. 152–170. Oxford: Oxford University Press.
- Pearson, R. G. and Dawson, T. P.** (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* **12**, 361–371.
- Piersma, T. and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228–233.
- Pigliucci, M.** (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore: Johns Hopkins University Press.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Development Core Team** (2009). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–94.
- R Development Core Team** (2014). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Richards, S.-A.** (1970). Physiology of thermal panting in birds. *Ann. Biol. Anim. Biophys.* **10**, 151–168.
- Ro, J. and Williams, J. B.** (2010). Respiratory and cutaneous water loss of temperate-zone passerine birds. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **156**, 237–246.
- Sabat, P., Cavieres, G., Veloso, C. and Canals, M.** (2006). Water and energy economy of an omnivorous bird: population differences in the rufous-collared sparrow (*Zonotrichia capensis*). *Comp. Biochem. Phys. A Mol. Integr. Physiol.* **144**, 485–490.
- Schlichting, C. D. and Pigliucci, M.** (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland: Sinauer Associates.
- Schmidt-Nielsen, K., Hainsworth, F. R. and Murrish, D. E.** (1970). Counter-current heat exchange in the respiratory passages: effect on water and heat balance. *Resp. Physiol.* **9**, 263–276.
- Smit, B. and McKechnie, A. E.** (2010). Avian seasonal metabolic variation in a subtropical desert: basal metabolic rates are lower in winter than in summer. *Funct. Ecol.* **24**, 330–339.
- Smit, B., Harding, C. T., Hockey, P. A. R. and McKechnie, A. E.** (2013). Adaptive thermoregulation during summer in two populations of an arid-zone passerine. *Ecology* **94**, 1142–1154.
- Swanson, D. L.** (2010). Seasonal metabolic variation in birds: functional and mechanistic correlates. *Curr. Ornithol.* **17**, 75–129.
- Tieleman, B. I. and Williams, J. B.** (1999). The role of hyperthermia in the water economy of desert birds. *Physiol. Biochem. Zool.* **72**, 87–100.
- Tieleman, B. I. and Williams, J. B.** (2000). The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiol. Biochem. Zool.* **73**, 461–479.
- Tieleman, B. I. and Williams, J. B.** (2002). Cutaneous and respiratory water loss in larks from arid and mesic environments. *Physiol. Biochem. Zool.* **75**, 590–599.
- Tieleman, B. I., Williams, J. B., Michaeli, G. and Pinshow, B.** (1999). The role of the nasal passages in the water economy of crested larks and desert larks. *Physiol. Biochem. Zool.* **72**, 219–226.
- Tieleman, B. I., Williams, J. B. and Buschur, M. E.** (2002a). Physiological adjustments to arid and mesic environments in larks (Alaudidae). *Physiol. Biochem. Zool.* **75**, 305–313.
- Tieleman, B. I., Williams, J. B., LaCroix, F. and Paillat, P.** (2002b). Physiological responses of houbara bustards to high ambient temperatures. *J. Exp. Biol.* **205**, 503–511.
- Tieleman, B. I., Williams, J. B. and Bloomer, P.** (2003a). Adaptation of metabolism and evaporative water loss along an aridity gradient. *Proc. R. Soc. B Biol. Sci.* **270**, 207–214.
- Tieleman, B. I., Williams, J. B., Buschur, M. E. and Brown, C. R.** (2003b). Phenotypic variation of larks along an aridity gradient: are desert birds more flexible? *Ecology* **84**, 1800–1815.
- Tieleman, B. I., van Noordwijk, H. J. and Williams, J. B.** (2008). Nest site selection in a hot desert: trade-off between microclimate and predation risk? *Condor* **110**, 116–124.
- Trost, C. H.** (1972). Adaptations of horned larks (*Eremophila alpestris*) to hot environments. *Auk* **89**, 506–527.
- Weathers, W. W.** (1981). Physiological thermoregulation in heat-stressed birds: consequences of body size. *Physiol. Zool.* **54**, 345–361.
- Webster, M. D. and Bernstein, M. H.** (1987). Ventilated capsule measurements of cutaneous evaporation in mourning doves. *Condor* **89**, 868.
- Whitfield, M. C., Smit, B., McKechnie, A. E. and Wolf, B. O.** (2015). Avian thermoregulation in the heat: scaling of heat tolerance and evaporative cooling capacity in three southern African arid-zone passerines. *J. Exp. Biol.* **218**, 1705–1714.
- Williams, J. B.** (1996). A phylogenetic perspective of evaporative water loss in birds. *Auk* **113**, 457–472.
- Williams, J. B.** (1999). Heat production and evaporative water loss of dune larks from the Namib Desert. *Condor* **101**, 432–438.
- Williams, J. B. and Tieleman, B. I.** (2000). Flexibility in basal metabolic rate and evaporative water loss among hoopoe larks exposed to different environmental temperatures. *J. Exp. Biol.* **203**, 3153–3159.
- Williams, J. B. and Tieleman, B. I.** (2005). Physiological adaptation in desert birds. *BioScience* **55**, 416–425.
- Withers, P. C.** (1992). *Comparative Animal Physiology*. Orlando: Saunders College Publishing.
- Wolf, B. O. and Walsberg, G. E.** (1996). Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. *J. Exp. Biol.* **199**, 451–457.